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EVALUATION OF FRUIT AND LEAF MORPHOLOGICAL VARIABILITY IN BEARBERRY (*Arctostaphylos uva-ursi* (L.) Spreng.) DINARIC ALPS POPULATIONS

Antonio VIDAKOVIĆ[1], Marijan VUKOVIĆ[1], Ana VUKOVIĆ[1], Matija MAGDIĆ[1], Valentina GAŠPAROVIĆ[1], Igor POLJAK[1]*

SUMMARY

The Dinaric Alps, including the Velebit Mountain in Croatia, are recognized for their rich plant biodiversity, including Arctic-alpine species like bearberry (*Arctostaphylos uva-ursi* (L.) Spreng., Ericaceae). This study investigates the morphological diversity of bearberry populations in the northern Dinaric Alps, focusing on fruit and leaf traits across three populations. Morphometric analysis of fruits and leaves revealed moderate variability, with leaves exhibiting higher variability than fruits. Significant correlations were found among both leaf and fruit traits, as well as between them, suggesting a linked growth pattern. Furthermore, contrary to our expectations of significant population differentiation due to the rugged montane terrain, 100% of the variability was attributed to within-population differences. This likely results from effective gene flow between populations, facilitated by wildlife species and traditional pastoral practices on the Velebit Mountain. Additionally, the lack of inter-population variability can be attributed to recolonization processes after the last glaciation, which suggest a shared regional origin of the studied populations. Although the studied populations originate from different altitudes, we did not detect a phenotypic plasticity with respect to this gradient. The lack of plasticity in the studied bearberry populations could be due to several reasons: the inherent stability of structural characteristics in response to environmental changes; similar soil and habitat conditions across the studied populations; and the reduced plasticity observed in alpine plants from higher altitudes due to extreme and stable environmental conditions. Overall, our study highlights the importance of preserving traditional land-use practices and enforcing legal protections within national and nature parks to conserve diversity and ensure the survival of bearberry populations. It underscores the role of human activities in enhancing plant population connectivity in alpine environments and calls for integrated conservation strategies that blend habitat protection with sustainable land use.

KEY WORDS: effective seed dispersal, morphometric analysis, phenotypic plasticity, population variability, conservation biology, endangered and rare species, Arctic-alpine plants, Dinaric Alps

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[1] Antonio Vidaković, PhD, Marijan Vuković, Ana Vuković, Matija Magdić, Valentina Gašparović, Assoc. Prof. Igor Poljak, PhD, University of Zagreb Faculty of Forestry and Wood Technology, Department of Forest Genetics, Dendrology and Botany, Zagreb, Croatia
* Corresponding author: Igor Poljak, email: ipoljak@sumfak.unizg.hr

INTRODUCTION

The Dinaric Alps (Dinarids) are a mountain range of southern and south-central Europe, usually recognized as one of the most important centres of European plant biodiversity and endemism (Médail and Diadema 2009, Médail et al. 2019, Liber et al. 2020). One of the largest mountains in this area is Velebit. Situated on the Adriatic coast of Croatia, it stretches about 145 km from the Vratnik pass above Senj in the northwest to the bend of the Zrmanja river in the southeast (Forenbacher 1990). The entire mountain area has been protected as a nature park, encompassing two national parks and one strict nature reserve. Velebit is home to about 2000 plant species, subspecies and varieties with a large number of rare, endangered, and protected plants (Topić et al. 2010), such as e.g. *Sibiraea laevigata* (L.) Maxim. and *Degenia velebitica* Hayek.

In higher altitudes and on Velebit summits, particularly prominent are Arctic-alpine (northern alpine) plants, persisted in this area since the Last Glacial Period (Birks and Willis 2008), such as e.g. *Arctostaphylos uva ursi* (L.) Spreng., *Dryas octopetala* L. and *Vaccinium vitis-idaea* L. Presently, the main part of those plants' distribution area is in the cold Arctic tundras, whereas in the south of Europe they only grow in high mountainous areas, where the climate is somewhat similar to the Arctic. Nowadays those plants in the southern mountainous areas are isolated because they are surrounded by a large area of what is for them an unfavourable habitat (Ronikier et al. 2023). In this isolation each group of plants lives independently, which often results in large differences between populations (Ægisdóttir et al. 2009, Bradburd et al. 2013). Today these high-mountain and Arctic plant species are considered to be particularly sensitive to climate change because of their specialized adaptation to the cold environment (Lesica and McCune 2004, Kougioumoutzis et al. 2021, Watts et al. 2022, Walas et al. 2023).

In the group of Arctic-alpine plants, because they are endangered and rare in the Dinaric area, we were particularly interested in the species *Arctostaphylos uva-ursi*, commonly known as bearberry. *A. uva-ursi* is a small procumbent woody groundcover shrub growing 5–15 cm high (Herman 1971, Forenbacher 1990). Its leaves are evergreen, leathery and shiny, oblanceolate to obovate in shape, sometimes narrowly elliptic, 1–3 long and 0.5–1 cm wide (Schütt 2008). Besides the leaves, this species is also characterized by bisexual, bell-shaped, white, pink, or pink-tipped flowers, and 0.6–0.8 cm large globose and red fruits (Idžojtić 2019). Flowering takes place in April and May, and the fruits ripen in August and September (Krüssmann 1962). The fruits persist on the plants into early winter, and are consumed by bears and many other animals. This species is widely distributed across circumboreal regions of the subarctic Northern Hemisphere (Schütt 2008), including North America, Europe and Asia. In the Dinaric area (Forenbacher 1990), there are isolated populations on karst mountainous pastures and glades, and in dwarf pine communities at altitudes above 1000 m. Due to excessive harvesting in nature, it has been placed on the Red List of Threatened Plants of Croatia, in the Vulnerable category (Marković et al. 2005), and it is strictly protected by law.

This species is best known for its leaves, which are the main natural source of arbutin (Asensio et al. 2020), used for centuries to treat urinary tract infections and other renal diseases. In addition, bearberry also has edible fruits (Schütt 2008). Raw and unprocessed fruits have an astringent and acidic taste, while cooking and drying makes them sweeter and more appetizing. Given the significance of this plant's leaves in folk medicine, there are numerous papers dealing with their phytochemical variability (Asensio et al. 2020, Shamilov et al. 2021, Sugier et al. 2021, Kravchenko et al. 2022). However, prior to the present study, little was known about the morphological variability of bearberry. In their paper, Remphrey et al. (1983) analysed the morphology and shrub architecture of bearberry in north Canada. Due to the variability of branch architecture and hairiness, as well as leaf hairiness, as many as 14 subspecies and varieties have been recognized, although nowadays they are considered synonyms by World Flora Online (WFO 2024).

In this study, we investigated the fruit and leaf diversity of *A. uva-ursi* populations in the northern area of the Dinaric Alps. Specifically, we asked three questions: (1) What is the morphological diversity of bearberry fruits and leaves in the studied area?; (2) Do the morphometric traits of fruits and leaves correlate with each other?; and (3) Are there any differences between the populations and shrubs within the populations, and is there any morphological structure within the species in the studied area?

MATERIALS AND METHODS

Sample collection

In October 2023, samples for leaf and fruit morphometric analyses were collected in three bearberry populations of northern Dinaric Alps (Figure 1, Table 1): P1–Zavižan; P2–Zečjak; P3–Kiza. For the localities situated within protected areas, permission to collect samples was obtained from the Ministry of Economy and Sustainable Development (UP/I 612-07/21-33/57). In each locality we selected ten plants with a spacing of at least 30 m between each individual, and collected sev-

Table 1 Populations, sampling sites, geographic coordinates, altitude and multivariate diversity index (MDI) for three studied bearberry populations.

Population ID	Population	Latitude	Longitude	Altitude (m)	MDI Index
P1	Zavižan	44.811461	14.973303	1557	4.164
P2	Zečjak	44.704138	14.972831	1340	3.736
P3	Kiza	44.545464	15.159640	1096	3.542

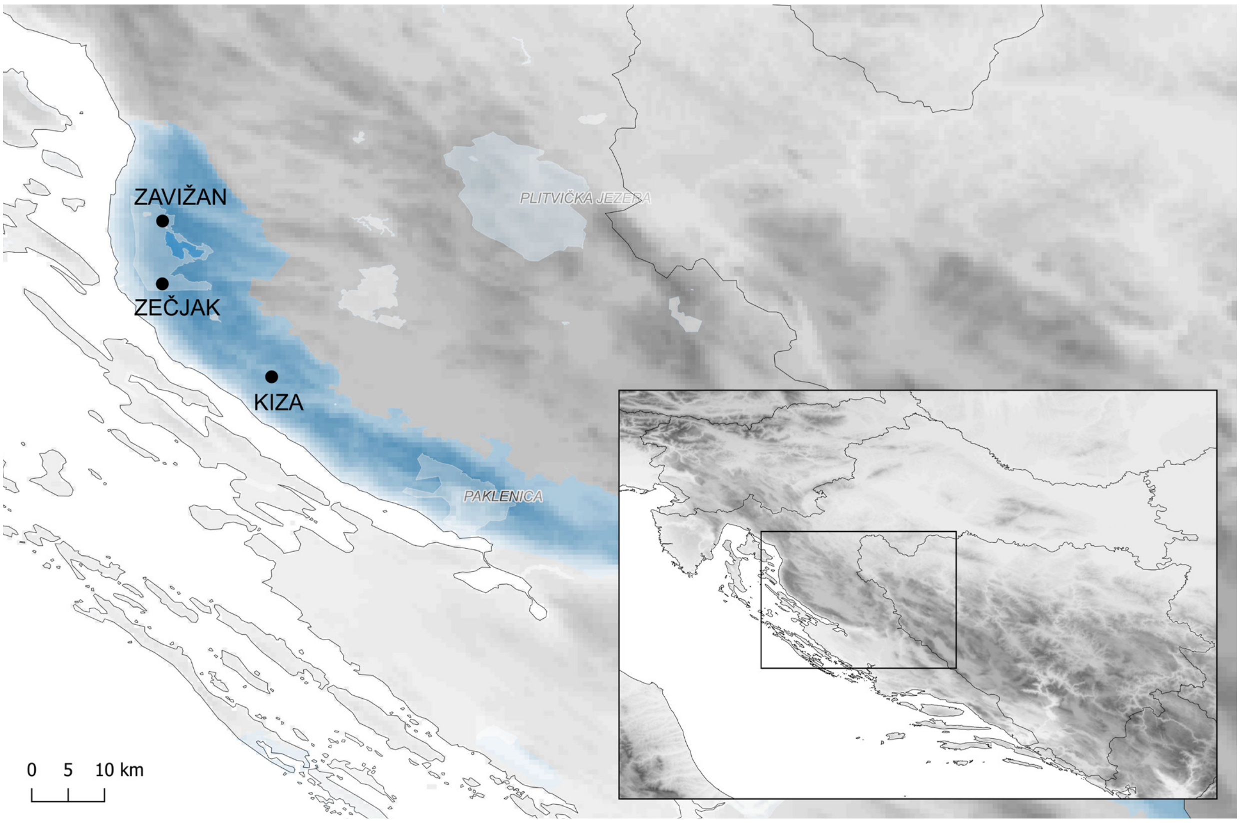

Figure 1 Geographic locations of the studied bearberry populations. Nature Park's borders are represented by darker shade of blue, while National Park's borders are depicted with light blue colour.

eral small branches from each plant with 20–30 mature and morphologically intact leaves. Once collected, the leaves were immediately placed in cardboard folders and transported back to the Herbarium DEND (University of Zagreb Faculty of Forestry and Wood Technology). From the same plants we also collected fruit samples for morphometric analysis, specifically 20 fruits from each shrub. The fruits were immediately (on the field) put into plastic zip-lock bags and placed into a portable cooler. Upon return to the lab, leaf samples were herbarized between newspaper sheets, and the fruits were stored in a refrigerator at 5˚C until measurement.

Morphometric analysis

Within five days of collection, the fruits were weighed (m), and their height (FH) and width (FW) were measured using a digital caliper (Alpha Tools®, Bahag AG, Germany). Furthermore, after the leaves were herbarized, 20 leaves were randomly selected for morphometric analysis. The leaves were then scanned using a flatbed scanner MICROTEK ScanMaker 4800 (Microtek International, Inc., Hsinchu, Taiwan), and the scanned images were analysed using WinFolia software package (WinFolia™ 2001) to obtain leaf morphological parameters. Finally, we obtained a total of eight leaf traits, including leaf area (LA), leaf perimeter (P), leaf length (LL), maximal leaf width (MLW), leaf length measured from the leaf base to the point of maximum leaf width (PMLW), leaf width at 90% of leaf length (LW90), petiole length (PL), and angle describing leaf base (LA10), i.e. the angle closed by the main leaf vein and the line connecting the leaf base to a set point on the leaf margin, at 10% of total leaf length. All leaf and fruit measurements were made with a precision of 0.01 mm. Based on the measured traits we also calculated three indices, two describing the shape of the leaf (MLW/LL and PMLW/LL) and one describing the shape of the fruit (FH/FW).

Statistical analysis

Following the procedure described in Sokal and Rohlf (2012), descriptive statistical parameters for all of the studied traits were calculated, including arithmetic mean, standard deviation, coefficient of variability, and minimal and maximal values. These parameters were calculated for each population and for the populations overall. Coefficients of variation for the studied fruit and leaf traits were interpreted as described in Depypere et al. (2007): CV < 10%, small variability; 10% < CV < 20%, normal variability; and CV > 25%, high variability.

To assess the possibility of conducting parametric tests, the symmetry, unimodality, and homoscedasticity of the data were verified (Sokal and Rohlf 2012). Assumptions of normality were checked using the Shapiro–Wilk test, and the assumption of homogeneity of variance was checked using Levene's test. The original data, which were nonhomoscedastic, prevented the direct utilization of parametric analyses. However, after standardization, the data exhibited a homogeneous level of variation, allowing us to use parametric tests. Ultimately, statistically significant differences between the studied populations and within populations were determined using hierarchical analysis of variance. The analyses were performed using the STATISTICA software package (Statistica 2018).

Pearson's correlation coefficients were calculated among all leaf and fruit traits including all studied individuals using the cor.test functions in R (R Core Team 2016). Strong correlations were considered those with *r* values equal to or greater than 0.7.

The principal component (PC) analysis was conducted in order to assess populations structure and to reveal the interactions between individuals and studied morphometric traits. The biplot was constructed by the first two principal components. The PC analysis was conducted using the "MorphoTools" R scripts in R v.3.2.2 (R Core Team 2016) by following the manual by Koutecký (2015).

The Euclidean distance matrix was calculated between all pairs of individuals based on the scores of the first two principal components (PC) considering 14 studied fruit and leaf traits. The average Euclidean distances were calculated for each population and used as the multivariate diversity index (MDI) of a population (Poljak et al. 2024a).

In addition, the Euclidean distance matrix was also used in the analysis of molecular variance (AMOVA, Excoffier et al. 1992) to partition the total morphological variance among and within populations. The significance levels of the variance components were determined after 10,000 permutations. The calculations were performed in Arlequin ver. 3.5.2.2 (Excoffier and Lischer 2010).

RESULTS

Descriptive statistics, i.e. means, standard deviations, minimal and maximal values and coefficient of variations of each morphological trait, are given in Table 2 for populations, and for the overall populations' sample. The coefficient of variations for the overall sample ranged from 4.2% to 24.5%. In terms of the traits pertaining only to fruits, only one has normal variability, namely fruit mass (m), while the other three variables (FH, FW and FH/FW) are characterized by low variability. Furthermore, the leaves were found to have nine out of ten studied traits with variability ranging from 11.5% to 24.4%, i.e. normal variability. The PMLW/LL variable exhibited variability under 10%.

Average fruit mass (m) for populations overall was 0.24 g, fruit height was 6.7 mm, and fruit width was 7.7 mm. The fruit length-to-width ratio was 0.87, ranging from 0.79 to 0.99. The highest average fruit mass (m) of 0.37 g was measured on a shrub from population Zečjak, and the lowest average of 0.15 g on a shrub from population Zavižan.

Average leaf blade length (LL) and width (MLW) were 1.5 cm and 0.7 cm, respectively. Leaf blade length (LL) and width (MLW) ranged from 1.06 to 1.95 cm, and from 0.57 to 0.95 cm, respectively. Leaf blade width at 90% of leaf blade length (LW90) was significantly lower than maximum leaf blade width (MLW), amounting to 0.45 cm. The leaf blade length/width ratio (MLW/LL) ranged from 0.34 to 0.59, and the ratio of length and distance from leaf blade base to the point of maximum leaf width (PMLW/LL) ranged from 0.55 to 0.66. The petiole (PL) was 0.43 cm long on average.

The results of the correlation analysis of the studied leaf and fruit morphological traits showed 57 significant correlations, out of 91 possible pairs (Table 3). The majority of those were positive, with only eight negative correlations found. Furthermore, out of the total number of correlations, only 17 demonstrated *r* values larger than 0.7.

When leaf traits are considered, 32 significant correlations were detected, among which 12 were strong (*r*<0.7). The largest number of correlations was observed for PMLW (nine) and LL (eight), and the lowest for LA10 (five) and PMLW/LL (three). Observing the fruit morphological traits, significant correlations were noted between all tested pairs except between fruit mass (m) and FH/FW. On the other hand, fruit mass (m) was strongly correlated with fruit width (FW) and fruit height (FH).

The analysis of leaf and fruit traits showed 20 significant correlations, all positive. No significant correlations were

Table 2 The results of the descriptive statistical analysis for the studied populations and morphometric traits. Fruit morphometric traits analysed: m–fruit mass (g); FH–fruit height (mm); FW–fruit width (mm); FH/FW–fruit height/fruit width. Leaf morphometric traits analysed: LA–leaf area (cm^2); P–leaf perimeter (cm); LL–leaf blade length (cm); MLW–maximum leaf width (cm); PMLW–leaf blade length measured from the leaf base to the point of maximum leaf width (cm); LW90–leaf blade width at 90% of the leaf blade length (cm); LA10–angle closed by the main leaf vein and the line defined by the leaf blade base and the point on the leaf margin, at 10% (°); PL–petiole length (cm); MLW/LL–maximum leaf width/leaf blade length; PMLW/LL–leaf blade length measured from the leaf base to the point of maximum leaf width/leaf blade length. Descriptive parameters: M–arithmetic mean, SD–standard deviation; Min-Max–range; and CV–coefficient of variation (%).

Trait	Descriptive parameters	Population			Total
		Zavižan	Zečjak	Kiza	
m	M±SD	0.22±0.06	0.25±0.06	0.24±0.06	0.24±0.06
	Min–Max	0.15–0.35	0.19–0.37	0.18–0.35	0.15–0.37
	CV (%)	28.06	22.88	23.82	24.45
FH	M±SD	6.68±0.77	6.69±0.43	6.71±0.49	6.69±0.56
	Min–Max	5.69–8.39	5.95–7.62	5.97–7.31	5.69–8.39
	CV (%)	11.51	6.49	7.35	8.42
FW	M±SD	7.51±0.61	7.86±0.76	7.80±0.61	7.73±0.66
	Min–Max	6.51–8.48	6.92–9.40	7.22–9.10	6.51–9.40
	CV (%)	8.18	9.70	7.85	8.58
FH/FW	M±SD	0.89±0.07	0.85±0.06	0.86±0.06	0.87±0.06
	Min–Max	0.80–0.99	0.79–0.95	0.80–0.97	0.79–0.99
	CV (%)	7.57	6.81	6.75	7.05
LA	M±SD	0.79±0.21	0.82±0.23	0.77±0.16	0.79±0.19
	Min–Max	0.49–1.17	0.59–1.28	0.52–0.97	0.49–1.28
	CV (%)	26.02	27.33	21.03	24.41
P	M±SD	3.68±0.53	3.81±0.44	3.87±0.55	3.79±0.50
	Min–Max	2.72–4.63	3.25–4.59	3.07–4.64	2.72–4.64
	CV (%)	14.27	11.66	14.12	13.09
LL	M±SD	1.48±0.22	1.55±0.17	1.59±0.24	1.54±0.21
	Min–Max	1.06–1.85	1.31–1.81	1.23–1.95	1.06–1.95
	CV (%)	14.63	10.73	15.24	13.56
MLW	M±SD	0.72±0.09	0.72±0.13	0.67±0.07	0.70±0.10
	Min–Max	0.61–0.88	0.60–0.95	0.57–0.77	0.57–0.95
	CV (%)	12.88	17.67	10.65	14.20
PMLW	M±SD	0.89±0.13	0.93±0.09	1.01±0.17	0.94±0.14
	Min–Max	0.63–1.12	0.80–1.07	0.78–1.27	0.63–1.27
	CV (%)	14.85	10.06	17.02	14.97
LW90	M±SD	0.46±0.06	0.45±0.08	0.43±0.04	0.45±0.06
	Min–Max	0.41–0.57	0.37–0.61	0.40–0.49	0.37–0.61
	CV (%)	12.32	18.71	8.78	13.72
LA10	M±SD	39.33±3.87	40.48±4.16	36.23±5.01	38.68±4.60
	Min–Max	34.11–45.60	33.75–46.30	27.80–43.90	27.80–46.30
	CV (%)	9.85	10.29	13.84	11.89
PL	M±SD	0.41±0.09	0.44±0.09	0.43±0.05	0.43±0.07
	Min–Max	0.28–0.60	0.31–0.61	0.34–0.56	0.28–0.61
	CV (%)	20.70	19.65	12.60	17.50
MLW/LL	M±SD	0.49±0.04	0.47±0.05	0.42±0.04	0.46±0.05
	Min–Max	0.45–0.59	0.40–0.56	0.34–0.47	0.34–0.59
	CV (%)	8.12	11.80	9.28	11.51
PMLW/LL	M±SD	0.60±0.02	0.60±0.02	0.63±0.02	0.61±0.03
	Min–Max	0.55–0.64	0.58–0.64	0.59–0.66	0.55–0.66
	CV (%)	3.73	3.01	3.45	4.16

Table 3 The results of correlation analysis between studied leaf and fruit traits. Morphometric traits' acronyms as in Table 2. *** significant at $p < 0.001$, ** significant at $0.001 < p < 0.01$, * significant at $0.01 < p < 0.05$, ns depicts non-significant values ($p > 0.05$).

Trait	m	FH	FW	FH/FW	LA	P	LL	MLW	PMLW	LW90	LA10	PL	MLW/LL	PMLW/LL
m		***	***	ns	***	***	***	**	***	*	ns	*	ns	ns
FH	0.772		***	*	***	***	***	**	***	**	ns	**	ns	ns
FW	0.955	0.652		*	**	**	**	*	**	*	ns	ns	ns	ns
FH/FW	-0.227	0.407	-0.425		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
LA	0.637	0.727	0.576	0.166		***	***	***	***	***	ns	***	ns	ns
P	0.683	0.741	0.586	0.182	0.915		***	***	***	***	*	***	ns	ns
LL	0.680	0.722	0.579	0.171	0.850	0.988		***	***	**	**	***	*	ns
MLW	0.506	0.584	0.464	0.125	0.933	0.730	0.629		**	***	ns	**	*	ns
PMLW	0.616	0.649	0.505	0.175	0.723	0.929	0.959	0.474		**	***	**	**	*
LW90	0.459	0.573	0.426	0.154	0.893	0.704	0.596	0.936	0.505		ns	**	*	ns
LA10	-0.228	-0.128	-0.087	-0.063	-0.071	-0.416	-0.508	0.146	-0.645	0.130		ns	***	***
PL	0.431	0.554	0.325	0.256	0.617	0.632	0.600	0.488	0.581	0.556	-0.171		ns	ns
MLW/LL	-0.195	-0.175	-0.118	-0.088	0.096	-0.292	-0.420	0.426	-0.551	0.392	0.738	-0.161		**
PMLW/LL	-0.019	-0.036	-0.081	0.061	-0.178	0.094	0.160	-0.344	0.430	-0.119	-0.625	0.134	-0.589	

detected between fruit traits and traits related to leaf shape, or between leaf traits and FW/FH, i.e. the trait describing the shape of the fruit.

The first three principal components had eigenvalues above 1 and accounted for 84.7% of total variability (Table 4). The first principal component (PC1) was highly positively correlated with nine leaf and fruit traits (P, LL, LA, PMLW, FH, m, MLW, LW90 and FW), all referring to the size of the leaf and fruit. The second principal component (PC2) was highly positively correlated with two leaf traits (MLW/LL and LA10), and highly negatively with one leaf trait (PMLW(LL), whereas the third principal component was highly positively correlated with one fruit trait (FH/FW). The traits highly positively or negatively correlated with the second and third principal component relate to the leaf and fruit shape. Furthermore, there was no clear grouping of individuals by population within the studied data.

At the level of individual traits, the populations differed in only one (MLW/LL) of the 14 studied morphological traits (Table 5). AMOVA demonstrated that the differences between the studied individuals within the populations account for 100% of the total variability. Furthermore, to determine the diversity within each of the studied populations, multivariate diversity index (MDI) values were calculated. The MDI values in the studied populations (Table 1), based on fruit and leaf morphological traits, ranged from 3.542 (P3–Kiza) to 4.164 (P3–Zavižan).

Table 4 Pearson's correlation coefficients between morphometric traits and scores of the first three principal components. Morphometric traits' acronyms as in Table 2.

Trait	PC–principal component		
	PC1	PC2	PC3
m	0.795	0.007	-0.521
FH	0.837	0.055	0.064
FW	0.700	0.095	-0.675
FH/FW	0.154	-0.068	0.884
LA	0.929	0.304	0.107
P	0.970	-0.094	0.075
LL	0.945	-0.221	0.043
MLW	0.770	0.569	0.130
PMLW	0.881	-0.428	0.069
LW90	0.762	0.487	0.197
LA10	-0.323	0.831	-0.008
PL	0.692	-0.025	0.258
MLW/LL	-0.202	0.915	0.070
PMLW/LL	0.069	-0.784	0.106
Eigenvalue	7.12	3.06	1.67
Variability (%)	50.87	21.83	11.96
Cumulative variability (%)	50.87	72.70	84.66

DISCUSSION

The leaf dimensions of *A. uva-ursi* obtained in this research were consistent with the data provided in the botanical literature (Krüssmann 1962, Schütt 2008). Specifically, the average leaf in Croatian bearberry populations measured 1.5 cm in length and 0.7 cm in width, which falls within the respective ranges of 1–3

Table 5 The results of the hierarchical analysis of variance. Morphometric traits' acronyms as in Table 2.

Trait	Components of the variance	F	p-value	Variability (%)
m	Among populations	0.55	0.58	0.00
	Within populations	47.39	0.00	66.17
	Error			33.83
FH	Among populations	0.00	1.00	0.00
	Within populations	34.90	0.00	58.82
	Error			41.18
FW	Among populations	0.81	0.45	0.00
	Within populations	41.14	0.00	63.09
	Error			36.91
FH/FW	Among populations	0.99	0.39	0.00
	Within populations	46.66	0.00	67.10
	Error			32.90
LA	Among populations	0.22	0.80	0.00
	Within populations	21.83	0.00	49.57
	Error			50.43
P	Among populations	0.35	0.71	0.00
	Within populations	22.81	0.00	50.97
	Error			49.03
LL	Among populations	0.72	0.50	0.00
	Within populations	24.05	0.00	53.03
	Error			46.97
MLW	Among populations	1.01	0.38	0.05
	Within populations	20.34	0.00	49.14
	Error			50.81
PMLW	Among populations	2.04	0.15	4.82
	Within populations	18.18	0.00	43.98
	Error			51.20
LW90	Among populations	0.44	0.65	0.00
	Within populations	13.88	0.00	38.16
	Error			61.84
LA10	Among populations	2.52	0.10	8.29
	Within populations	27.80	0.00	52.52
	Error			39.19
PL	Among populations	0.33	0.73	0.00
	Within populations	16.17	0.00	41.90
	Error			58.10
MLW/LL	Among populations	6.33	0.01	15.75
	Within populations	18.32	0.00	41.78
	Error			42.48
PMLW/LL	Among populations	2.67	0.08	9.62
	Within populations	4.93	0.00	14.34
	Error			73.04

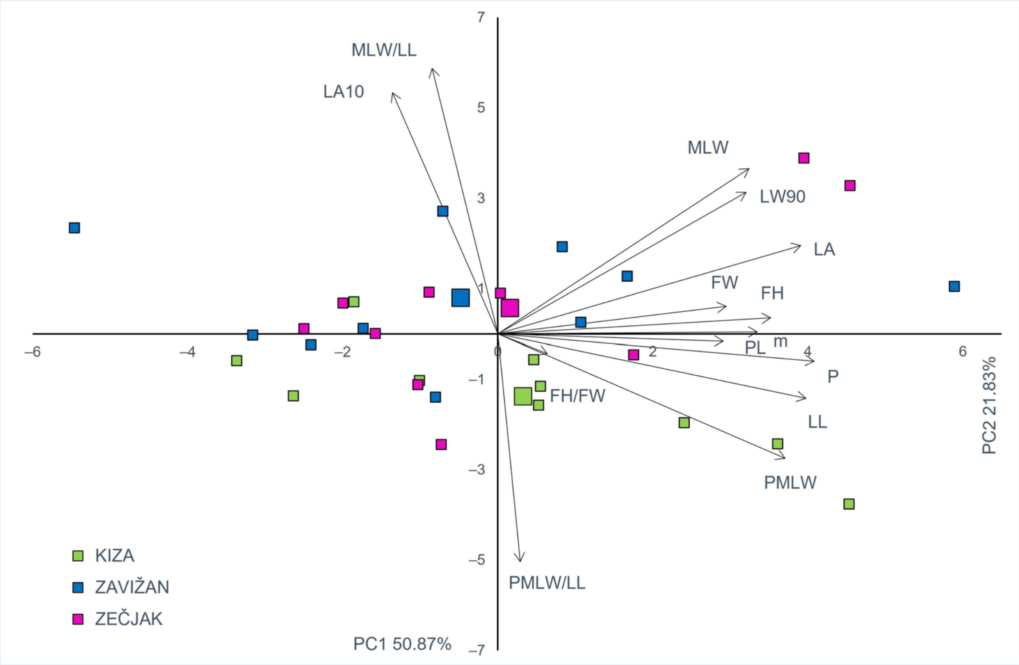

Figure 2 Biplot of the principal component analysis (PCA) based on 14 fruit and leaf morphometric traits in the studied bearberry populations. Each individual shrub is indicated by a small sign, while the population barycenters are represented by larger ones. Morphometric traits' acronyms as in Table 2.

cm and 0.5–1 cm listed by the mentioned authors. The same applies to the dimensions of the fruits, which, with an average height of 6.7 mm, fall within the range of 6–8 mm specified by Schütt (2008) and Idžojtić (2019). Furthermore, bearberry demonstrated moderate coefficients of variability, ranging from 7.1 to 24.5% in fruit morphology, and from 4.2 to 24.4% in leaf morphology. This represents approximately half the variability of another Arctic-alpine species, *Dryas octopetala*, whose variability of comparable traits ranged from 27.6 to 56.2% (Marcysiak 2014).

Significant correlations were found between both fruit and leaf morphometric traits. These findings align with general expectations, as it is well-known that when one dimension of a fruit or leaf increases, other related dimensions also tend to increase, indicating the linked nature of their development. Furthermore, significant correlations were also found between bearberries' leaf and fruit morphological traits, particularly those related to leaf and fruit size. In other words, shrubs with smaller leaves had smaller fruits, and vice versa. Such correlation analyses are rarely conducted and often result in no significant correlations or only weak ones (Poljak et al. 2024b). Nonetheless, our results align with the findings of Fishler et al. (1983), who highlight leaf area as a crucial factor influencing fruit growth. A larger photosynthetically active leaf area could contribute more to better fruit development, ultimately affecting fruit size.

The finding that bearberry populations on Velebit do not differ significantly is indeed unexpected, given the typical genetic differentiation observed in alpine landscapes with high mountain ridges and steep valleys, which often limit gene flow among plant populations (Cain et al. 2000, Theurillat and Guisan 2001). This usually results in stronger genetic differentiation among populations compared to more homogeneous landscapes (Till-Bottraud and Gaudeul 2002). Alternatively, heterogeneity across small spatial scales in these habitats could also favour adaptive phenotypic plasticity (Alpert and Simms 2002, Hamann et al. 2016). However, in our study, 100% of the morphological variability was attributed to within-population variability. This suggests effective and strong gene flow between populations, which in the case of the barberry populations can be explained by the movement of the wildlife, such as deer, bears, and various small mammals and bird species, which feed on bearberry fruits (Crane 1991), and the historical and ongoing pastoral movements on the Velebit Mountain (Alegro and Šegota 2019). This human-mediated seed dispersal, along the wildlife gene flow, could counteract the population isolation typically caused by the rugged alpine terrain. In general, such effective gene flow ensures a healthy and resilient ecosystem by maintaining genetic diversity within plant populations, which is essential for their long-term survival and adaptability (Ellstrand and Elam 1993, Frankham 1996).

Furthermore, another possible explanation for the lack of differentiation between the studied populations might stem from their evolutionary history and recolonization processes after the last glaciation. Considering their geographical proximity, it is safe to assume they originated from the same refugial area. Comparable patterns of limited population differentiation, or more precisely, the absence of significant differentiation, have also been observed in other woody species, such as *Juniperus deltoides* R.P.Adams (Brus et al. 2010) and *Salix reticulata* L. (Marcysiak 2012).

Contrary to the assumption that altitude affects plant morphology (Körner et al. 1989, Cruz-Maldonado et al. 2021), our research shows that the bearberry populations we studied did not show any variations in their leaf and fruit morphology along the altitudinal gradient. This lack of phenotypic plasticity could be due to several reasons. Firstly, such a small, procumbent shrub with evergreen and small leaves may exhibit less morphological plasticity compared to physiological traits due to the inherent stability of structural characteristics in response to environmental changes (Arnold et al. 2022). Additionally, we cannot exclude the possibility that some morpho-anatomical traits, such as leaf thickness and stomatal density, in bearberry populations that we studied change with altitude, which has been previously reported in alpine plants (Bertel et al. 2022). Furthermore, similar soil conditions in mountainous pastures where these populations are located may have favoured the same phenotypes and the absence of phenotypic plasticity. Such consistent environmental conditions across different altitudes could lead to uniform selection pressure, resulting in similar leaf and fruit morphological traits despite the variation in altitude. Finally, studies on alpine plants, such as *Arabis alpina* L. (de Villemereuil et al. 2018), have indicated that populations from higher altitudes often exhibit reduced phenotypic plasticity compared to those from lower altitudes. This reduced plasticity could be explained by the extreme and stable environmental conditions at higher altitudes, which favour more specialized and less plastic traits. Nevertheless, although we did not find variations in leaf and fruit morphology along the altitudinal gradient, we did observe that population diversity changes with altitude. Specifically, the population from the lowest elevation exhibited the least variability, while the population from the highest elevation showed the greatest diversity.

Along with effective gene flow and the size of bearberry populations, an additional factor that positively affects the maintenance of high diversity within populations is the fact that these populations are located within national parks and nature parks. Protected areas are known to play a key role in biodiversity conservation and in reducing exploitation of the species of interest, leading to greater species richness and its abundance (Gray et al. 2016, Cavalcante de Souza and Prevedello 2020). The legal protection helps to ensure the conservation of bearberry's genetic diversity, which is crucial for its long-term survival. Protecting such species in their natural habitats is essential for maintaining overall biodiversity and ecosystem stability (Hanjalić Kurtović et al. 2025). In general, the protection of mountain plants involves preserving their natural habitats, preventing habitat fragmentation, and mitigating the impacts of climate change. As the results of our study indicate, conservation efforts should also focus on maintaining traditional land-use practices that support biodiversity, such as sustainable grazing. Legal protection, public awareness, and scientific research including long-term monitoring are essential components in the effective conservation of these species.

CONCLUSIONS

The study revealed several important conclusions regarding the morphological variability of bearberry populations in the Dinaric Alps. Firstly, the observed morphological variability in fruit and leaf traits was generally moderate. This moderate variability aligns with previous studies on similar species in different regions, suggesting a stable pattern of morphological variability within this species. Significant correlations were identified among both fruit and leaf traits, as well as between them, which supports the notion that leaf area significantly influences fruit development, highlighting the connection of vegetative growth and reproductive success. No significant differences were found among populations, as all variability was being attributed to within-population differentiation. This unexpected pattern of morphological variability could be explained by several factors: the effective gene flow facilitated by the movement of wildlife and human-mediated seed dispersal through traditional pastoral practices; the evolutionary history and recolonization processes after the last glaciation; and the absence of phenotypic plasticity in structural traits. All in all, our study emphasizes the importance of conservation efforts in maintaining species diversity and ecosystem stability. The legal protection of bearberry populations within the Northern Velebit National Park and Velebit Nature Park appears to play a crucial role in preserving the diversity of bearberry populations.

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REFERENCES

- Ægisdóttir, H.H., P. Kuss, J. Stöcklin, 2009: Isolated populations of a rare alpine plant show high genetic diversity and considerable population differentiation. Annals of Botany 104 (7): 1313–1322. https://doi.org/10.1093/aob/mcp242.
- Alegro, A., V. Šegota, 2019: Diversity and endangerment of the grasslands of the Northern Velebit National park. Senjski zbornik 46: 61–80. https://doi.org/10.31953/sz.46.1.3.
- Alpert, P., E.L. Simms, 2002: The relative advantages of plasticity and fixity in different environments: when is it good for a plant to adjust? Evolutionary Ecology 16: 285–297. https://doi.org/10.1023/A:1019684612767.
- Arnold, P.A., S. Wang, A.B. Nicotra, A.A. Catling, L.E.B. Kruuk, 2022: Patterns of phenotypic plasticity along a thermal gradient differ by trait type in an alpine plant. Functional Ecology 36 (1): 141–152. https://doi.org/10.1111/1365-2435.14128.
- Asensio, E., D. Vitales, I. Pérez, L. Peralba, J. Viruel, C. Montaner, J. Vallès, T. Garnatje, E. Sales, 2020: Phenolic compounds content and genetic diversity at population level across the natural distribution range of bearberry (*Arctostaphylos uva-ursi*, Ericaceae) in the Iberian Peninsula. Plants 9: 1250. https://doi.org/10.3390/plants9091250.
- Bertel, C., D. Kaplenig, M. Ralser, E. Arc, F. Kolář, G. Wos, K. Hülber, A. Holzinger, I. Kranner, G. Neuner, 2022: Parallel differentiation and plastic adjustment of leaf anatomy in Alpine *Arabidopsis arenosa* Ecotypes. Plants 11 (19): 2626. https://doi.org/10.3390/plants11192626.
- Birks H.J.B., K.J. Willis, 2008: Alpines, trees, and refugia in Europe. Plant Ecology & Diversity 1 (2): 147–160. https://doi.org/10.1080/17550870802349146.
- Bradburd, G.S., P.L. Ralph, G.M. Coop, 2013: Disentangling the effects of geographic and ecological isolation on genetic differentiation. Evolution 67 (11): 3258–3273. https://doi.org/10.1111/evo.12193.
- Brus, R., D. Ballian, P. Zhelev, M. Pandža, M. Bobinac, J. Acevski, Y. Raftoyannis, K. Jarni, 2010: Absence of geographical structure of morphological variation in *Juniperus oxycedrus* L. subsp. *oxycedrus* in the Balkan Peninsula. European Journal of Forest Research 130 (4): 657–670. https://doi.org/10.1007/s10342-010-0457-1
- Cain, M.L., B.G. Milligan, A.E. Strand, 2000: Long-distance seed dispersal in plant populations. American Journal of Botany 87 (9): 1217–1227. https://doi.org/10.2307/2656714.
- Cavalcante de Souza, A., J.A. Prevedello, 2020: The importance of protected areas for overexploited plants: Evidence from a biodiversity hotspot. Biological Conservation 243: 108482. https://doi.org/10.1016/j.biocon.2020.108482.
- Crane, M.F., 1991: *Arctostaphylos uva-ursi*. In: Fire Effects Information System, U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory. https://www.fs.usda.gov/database/feis/plants/shrub/arcuva/all.html
- Cruz-Maldonado, N., M. Weemstra, L. Jiménez, C. Roumet, G. Angeles, I. Barois, M. de los Santos, M.A. Morales-Martinez, R.A. Palestina, H. Rey, K. Sieron, A. Stokes, F. Anthelme, 2021: Aboveground-trait variations in 11 (sub)alpine plants along a 1000-m elevation gradient in tropical Mexico. Alpine Botany 131: 187–200. https://doi.org/10.1007/s00035-021-00260-z.
- de Villemereuil, P., I. Till-Bottraud, M. Mouterde, O.E. Gaggiotti, 2018: Patterns of phenotypic plasticity and local adaptation in the wide elevation range of the alpine plant *Arabis alpine*. Journal of Ecology 106 (5): 1952–1969. https://doi.org/10.1111/1365-2745.12955
- Depypere, L., P. Chaerle, K.V. Mijnsbrugge, P. Goetghebeur, 2007: Stony endocarp dimension and shape variation in *Prunus* section *Prunus*. Annals of Botany 100 (7): 1585–1597. https://doi.org/10.1093/aob/mcm260.
- Ellstrand, N.C., D.R. Elam, 1993: Population genetic consequences of small population size: Implications for plant conservation. Annual Review of Ecology, Evolution, and Systematics 24: 217–242. http://dx.doi.org/10.1146/annurev.es.24.110193.001245.
- Excoffier, L., H.E.L. Lischer, 2010: Arlequin suite ver. 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10 (3): 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x.
- Excoffier, L., P.E. Smouse, J.M. Quattro, 1992: Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitocondrial DNA restriction sites. Genetics 131 (2): 479–491. https://doi.org/10.1093/genetics/131.2.479.
- Fishler, M., E. Goldschmidt, S.P. Monselise, 1983: Leaf area and fruit size on gridled grapefruit branches. Journal of the American Society for Horticultural Science 108 (2): 218–221. https://doi.org/10.21273/JASHS.108.2.218.
- Forenbacher, S., 1990: Velebit i njegov biljni svijet. Školska knjiga, Zagreb.
- Frankham, R., 1996: Relationship of genetic variation to population size in wildlife. Conservation Biology 10 (6): 1500–1508. https://doi.org/10.1046/j.1523-1739.1996.10061500.x.
- Gray, C., S. Hill, T. Newbold, L.N. Hudson, L. Börger, S. Contu, A.J. Hoskins, S. Ferrier, A. Purvis, J.P.W. Scharlemann, 2016: Local biodiversity is higher inside than outside terrestrial protected areas worldwide. Nature Communications 7: 12306. https://doi.org/10.1038/ncomms12306.
- Hamann, E., H. Kesselring, G.F.J. Armbruster, J.F. Scheepens, J. Stöcklin, 2016: Evidence of local adaptation to fine- and coarse-grained environmental variability in *Poa alpina* in the Swiss Alps. Journal of Ecology 104 (6): 1620–1630. https://doi.org/10.1111/1365-2745.12628.
- Hanjalić Kurtović, J., B. Kalamujić Stroil, S. Siljak-Yakovlev, N. Pojskić, A. Durmić-Pašić, A. Hajrudinović-Bogunić, L. Lasić, L. Ušanović, F. Bogunić, 2025: Spatial distribution of genetic, ploidy, and morphological variation of the edaphic steno-endemic *Alyssum moellendorfianum* (Brassicaceae) from the Western Balkans. Plants 14: 146. https://doi.org/10.3390/plants14020146.
- Herman, J., 1971: Šumarska dendrologija. Stanbiro, Zagreb.
- Idžojtić, M., 2019: Dendrology: Cones, flowers, fruits and seeds. Elsevier-Academic Press, London, San Diego, Cambridge, Oxford.
- Körner, C., M. Neumayer, S. Menendez-Riedl, A. Smeets-Scheel, 1989: Functional morphology of mountain plants. Flora 182: 353–383. https://doi.org/10.1016/S0367-2530(17)30426-7
- Kougioumoutzis, K., I.P. Kokkoris, A. Strid, T. Raus, P. Dimopoulos, 2021: Climate-change impacts on the southernmost Mediterranean Arctic-Alpine plant populations. Sustainability 13 (24): 13778. https://doi.org/10.3390/su132413778.
- Koutecký, P., 2015: MorphoTools: A set of R functions for morphometric analysis. Plant Systematics and Evolution 301: 1115–1121. https://doi.org/10.1007/s00606-014-1153-2
- Kravchenko, G., O. Krasilnikova, A. Raal, M. Mazen, N. Chaika, I. Kireyev, A. Grytsyk, O. Koshovyi, 2022: *Arctostaphylos uva-ursi* L. leaves extract and its modified cysteine preparation for the management of insulin resistance: chemical analysis and bioactivity. Natural Products and Bioprospecting 12 (1): 30. https://doi.org/10.1007/s13659-022-00352-1.
- Krüssmann, G., 1962: Handbuch der Laubgehölze. Paul Parkey Verlag, Berlin.
- Lesica, P., B. McCune, 2004: Decline of arctic-alpine plants at the southern margin of their range following a decade of climatic warming. Journal of Vegetation Science 15 (5): 679–690. https://doi.org/10.1111/j.1654-1103.2004.tb02310.x.

- Liber, Z., B. Surina, T. Nikolić, D. Škrtić, Z. Šatović, 2020: Spatial distribution, niche ecology and conservation genetics of *Degenia velebitica* (Brassicaceae), a narrow endemic species of the north-western Dinaric Alps. Plant Systematics and Evolution 306: 64. https://doi.org/10.1007/s00606-020-01695-3.
- Marcysiak, K., 2012: Diversity of *Salix reticulata* L. (Salicaceae) leaf traits in Europe and its relation to geographical position. Plant Biosystems 146: 101–111. https://doi.org/10.1080/11263504.2012.727879
- Marcysiak, K., 2014: Geographical differentiation of *Dryas octopetala* in Europe based on morphological features. Dendrobiology 72: 113–123. http://dx.doi.org/10.12657/denbio.072.010.
- Marković, Lj., Z. Liber, M. Palković, 2005: *Arctostaphylos uva-ursi* (L.) Spreng. (HRCP002605). Crveni popis divljih vrsta Hrvatske, Zavod za zaštitu okoliša i prirode Ministarstva gospodarstva i održivog razvoja, Zagreb.
- Médail, F., A.C. Monnet, D. Pavon, T. Nikolić, P. Dimopoulos, G. Bacchetta, J. Arroyo, Z. Barina, M.C. Albassatneh, G. Domina, B. Fady, V. Matevski, S. Mifsud, A. Leriche, 2019: What is a tree in the Mediterranean Basin hotspot? A critical analysis. Forest Ecosystems 6: 17. https://doi.org/10.1186/s40663-019-0170-6.
- Médail, F., K. Diadema, 2009: Glacial refugia influence plant diversity patterns in the Mediterranean Basin. Journal of Biogeography 36: 1333–1345. https://doi.org/10.1111/j.1365-2699.2008.02051.x.
- Poljak, I., Z. Šatović, A. Vidaković, K. Tumpa, M. Idžojtić, 2024a: Population variability of rosemary willow (*Salix eleagnos* Scop.) based on the leaf morphometry: Evidence of small and large-leaf morphotypes. Šumarski list 148 (5–6): 219–235. https://doi.org/10.31298/sl.148.5-6.1.
- Poljak, I., A. Vidaković, L. Benić, K. Tumpa, M. Idžojtić, Z. Šatović, 2024b: Patterns of leaf and fruit morphological variation in marginal populations of *Acer tataricum* L. subsp. *tataricum*. Plants 13: 320. https://doi.org/10.3390/plants13020320.
- R Core Team, 2016: R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org/
- Remphrey, W.R., T.A. Steeves, B.R. Neal, 1983: The morphology and growth of *Arctostaphylos uva-ursi* (bearberry): An architectural analysis. Canadian Journal of Botany 61 (9): 2430–2450. https://doi.org/10.1139/b83-267
- Ronikier, M., N. Kuzmanović, D. Lakušić, I. Stevanoski, Z. Nikolov, N.E. Zimmermann, 2023: High-mountain phylogeography in the Balkan Peninsula: Isolation pattern in a species of alpine siliceous grasslands and its possible background. Alpine Botany 133: 101–115. https://doi.org/10.1007/s00035-023-00296-3.
- Schütt, P., U.M. Lang, 2008: Enzyklopädie der Holzgewächse, Wiley-VCH, Weinheim.
- Shamilov, A.A., V.N. Bubenchikova, M.V. Chernikov, D.I. Pozdnyakov, E.R. Garsiya, M.V. Larsky, 2021: Bearberry (*Arctostaphylos uva-ursi* (L.) Spreng.): chemical content and pharmacological activity. Journal of Experimental Food Chemistry 12 (3): 49–66.
- Sokal, R.R., F.J. Rohlf, 2012: Biometry: The principles and practice of statistics in biological research. W.H. Freeman and Co., New York.
- Statistica, 2018: Version 13, TIBCO Software Inc., Palo Alto. http://www.statsoft.com.
- Sugier, P., Ł. Sęczyk, D. Sugier, R. Krawczyk, M. Wójcik, J. Czarnecka, S. Okoń, A. Plak, 2021: Chemical characteristics and antioxidant activity of *Arctostaphylos uva-ursi* L. Spreng. at the Southern border of the geographical range of the species in Europe. Molecules 26 (24): 7692. https://doi.org/10.3390/molecules26247692.
- Theurillat, J.P., A. Guisan, 2001: Potential impact of climate change on vegetation in the European Alps: A review. Climatic Change 50: 77–109. http://dx.doi.org/10.1023/A:1010632015572.
- Till-Bottraud, I., M. Gaudeul, 2002: Intraspecific genetic diversity in Alpine plants. In (Körner, C., E.M. Spehn, eds.): Mountain biodiversity: A global assessment. Parthenon Publishing, New York, pp. 23–34.
- Topić J., T. Nikolić, N. Vuković, 2010: Velebit – Botanički važna područja Hrvatske (Botanically relevant areas of Croatia). Sveučilište u Zagrebu Prirodoslovno-matematički fakultet, Školska knjiga, Zagreb.
- Walas, Ł., M. Pietras, M. Mazur, Á. Romo, L. Tasenkevich, Y. Didukh, A. Boratyński, 2023: The perspective of Arctic–Alpine species in southernmost localities: The example of *Kalmia procumbens* in the Pyrenees and Carpathians. Plants 12 (19): 3399. https://doi.org/10.3390/plants12193399.
- Watts, S.H., D.K. Mardon, C. Mercer, D. Watson, H. Cole, R.F. Shaw, A.S. Jump, 2022: Riding the elevator to extinction: Disjunct arctic-alpine plants of open habitats decline as their more competitive neighbours expand. Biological Conservation 272: 109620. https://doi.org/10.1016/j.biocon.2022.109620.
- WFO, 2024: World Flora Databse Online. https://wfoplantlist.org/
- WinFolia^{TM}, 2001: version PRO 2005b. Regent Instruments Inc., Quebec City.